

Total and Water-Soluble Phosphorus in Broiler Litter over Three Flocks with Alum Litter Treatment and Dietary Inclusion of High Available Phosphorus Corn and Phytase Supplementation

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ABSTRACT Three pen trials were conducted to determine the main effect of alum addition to litter on form of poultry litter P using a 2 × 2 factorial structure of the subunit treatments: diets including high available phosphorus/low phytate corn (HAPC) and phytase (PHYT). Male broilers (1,760 per flock) were grown to 42 d having starter diets with 0.45% available P and grower diets with 0.35% available P. In the first trial, total litter P (tP) was greatest for the yellow dent corn (YDC) diet (12 g/kg) and least for the HAPC and PHYT combination (H&P) diet (6.9 g/kg) with the individual PHYT and HAPC diets falling in between at 9.1 g/kg and 9.4 g/kg tP. Also in the first trial, the litter water-soluble P (wP) was highest

for PHYT (2.8 g/kg), least for the HAPC and H&P diets (1.5 g/kg) with the YDC diet falling between (2.2 g/kg). Alum was added to the litter after the first experiment. In the second and third experiments, alum inclusion significantly reduced the wP when compared with the treatments with no alum. In the third trial, the least wP was present in the alum-HAPC treatment. Phytase, YDC, and HAPC diets with no alum litter treatment generated the most wP. Since these diets appear to have little or no difference with respect to quantity of wP, this work suggests that form of litter P generated by alternative diets should be considered as criteria when attempting to reduce P in broiler litter applied to land.

(*Key words:* alum, broiler, high available phosphorus corn, phosphorus, phytase)

2003 Poultry Science 82:1544–1549

INTRODUCTION

Litter management issues related to excess P levels in litter, especially water-soluble P (wP), may soon encumber the poultry industry. Proper management of this valuable resource is key to maintaining industry viability while promoting environmental stewardship in areas of the U.S. where poultry production is concentrated. Runoff and soil erosion can carry excess P applied to land into natural waterways causing eutrophication (the over abundance of nutrients in surface waters). The P in runoff is largely a soluble form, the type most available to aquatic plants (Moore et al., 1999). When too much P is available to these plants, they can proliferate and upon decomposition deprive aquatic life of oxygen. Under eutrophic conditions some algae produce *Pfiesteria* that have caused

lesions on fish and resulted in fish kills in rivers around the Chesapeake Bay and mid-Atlantic region of the U.S. (Silverstein, 1999). On the other hand, several benefits are associated with the use of broiler litter as fertilizer. Kelling et al. (1995) showed that significant increases in grain and corn silage yields occur when using fresh poultry litter as opposed to composted. Poultry litter as a soil amendment generally improves tillage, reduces compaction, provides more organic matter and nutrients, and thus increases forage productivity (Van Dyne and Gilbertson, 1978; Edmisten et al., 1992).

Alum treatment of broiler litter has been shown to reduce wP levels in poultry litter as well as control ammonia volatilization (Moore et al., 1995a). Huff et al. (1996) evaluated the potential toxicity of alum to chicks and found a correlation but at levels beyond those expected

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Received for publication December 19, 2002.

Accepted for publication May 13, 2003.

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Abbreviation Key: aP = available P; F:G = feed to gain; HAPC = high available phosphorus corn; H&P = high available phosphorus corn and phytase combination diet; PHYT = phytase diet; tP = total litter P; wP = water-soluble P; YDC = yellow dent corn.

from litter consumption. In a review of alum effects on ammonia loss and P runoff from broiler litter, Moore et al. (1999) reported higher body weights, lower mortality, and better feed conversion for broilers on alum-treated litter. The literature is sparse in addressing alum treatment of litter in which broilers have consumed alternative diets, such as including high available phosphorus corn (HAPC) or phytase supplementation or both.

Modified broiler diets, such as the inclusion of HAPC or phytase enzyme, are viable methods for reducing the amount of phosphorus excreted in conventional wood shavings litter (Sebastian et al., 1998; Stilborn, 1998). During the 1990s, USDA scientists exploited a beneficial corn mutation providing low phytate P and high available P levels (Raboy and Gerbasi, 1996). Using the low phytic acid 1-1 allele of the corn LPA1 gene, a major seed company² has produced hybrid seed that produces high available phosphorus grain referred to as HAPC in this paper. This hybrid contains approximately 0.27% total dietary P of which about 0.17% is available to the broiler; normal yellow dent corn (YDC) has a similar level of total dietary P but only about 0.03% that is available (Waldroup et al., 2000; Yan et al., 2000). Consequently, P excretion is reduced using HAPC because less phytate-bound P is supplied to the broiler. Nonruminants, such as chickens, lack the quantity of endogenous phytase enzymes needed to break down phytate P (Kornegay, 1996). The efficiency of phytase to increase the bioavailability of phytate P was demonstrated by Nelson et al. (1971) and has been shown capable of maintaining performance (Sohail and Roland, 1999; Scheideler and Ferket, 2000) and reducing litter P as long as dietary inorganic supplements were reduced accordingly (Denbow et al., 1998; Ibrahim et al., 1999). Hatten et al. (2001) reported improved bone ash content, body weight gain, and tibia length with broiler dietary phytase supplementation. A review article regarding phytate P and phytase in poultry nutrition has been published by Sebastian et al. (1998).

In one of the few studies growing broilers to market weights, Yan et al. (2000) evaluated YDC and HAPC in combination with phytase enzyme and reduced levels of dietary P. They reported fecal total P was reduced without sacrificing live performance and skeletal integrity. The unanswered question remains as to how these dietary modifications, in conjunction with litter amendments, affect wP, as well as what changes occur with subsequent flocks. Water-soluble P concentrations possess the most eutrophication threat for waterways. This research was conducted to determine the effect of alum litter treatment on the quantity of wP over continuous flocks with broilers fed diets that meet National Research Council (1994) starter and grower requirements and that include HAPC and phytase enzyme.

TABLE 1. Percentage of nutrient values of normal yellow dent corn (YDC) and high available phosphorus corn (HAPC) on as-is basis¹

Nutrient (%)	YDC	HAPC
Dry matter	88.87	86.22
CP	9.69	8.75
Crude fiber	1.90	1.60
Ash	1.41	1.29
Crude fat	3.67	3.01
Ca	0.009	0.006
Total P	0.285	0.270
Phytate, P	0.269	0.084
Nonphytate P	0.016	0.186
Alanine	0.64	0.56
Arginine	0.39	0.37
Aspartic acid	0.55	0.48
Cystine	0.20	0.19
Glutamic acid	1.68	1.38
Glycine	0.33	0.27
Histidine	0.23	0.22
Isoleucine	0.30	0.27
Leucine	1.06	0.90
Lysine	0.29	0.27
Methionine	0.21	0.18
Phenylalanine	0.42	0.36
Proline	0.76	0.64
Serine	0.39	0.34
Threonine	0.29	0.27
Tyrosine	0.15	0.14
Tryptophan	0.08	0.07
Valine	0.40	0.38

¹Values provided by Pioneer Hi-Bred International, Inc., Johnston, IA.

MATERIALS AND METHODS

Dietary Formulations

Diets were formulated to be isocaloric and isonitrogenous based on nutrient values for the YDC and HAPC (Table 1). The corn caloric contents used to formulate the diets were similar and were assumed to be 3,394 ME/kg. Starter diets (1 to 21 d) and grower diets (22 to 42 d) provided 0.45 and 0.35% available P (aP) to meet NRC (1994) requirements. Phytase supplementation replaced 0.1% dietary available P in the corn-soybean diets in which the enzyme was included (Mitchell and Edwards, 1996). Four diets (Table 2) resulted: 1) NRC requirement for aP (YDC) diet; 2) HAPC corn formulation to meet NRC aP (HAPC); 3) YDC + phytase formulation to meet NRC aP minus 0.1% (PHYT); and 4) HAPC + phytase formulation to meet NRC requirements aP minus 0.1% (H&P). Phytase enzyme³, where used, was added at 600 phytase units⁴/kg. Enzyme activity in the feed (after mixing) was measured by the manufacturer. All feed was in mash form.

General Procedures

The experimental design was a split-plot in which the main unit design was a randomized complete block with four replications. The main unit treatment was alum addition (0.091 kg/bird) to the litter; the subunit treatments were HAPC and phytase supplementation in a 2 × 2 factorial structure for the diets given above. Male, Ross ×

²Pioneer Hi-Bred International, Inc., Johnston, IA.

³Natuphos, BASF Corporation, Mt. Olive, NJ.

⁴One phytase unit is the activity of phytase that generates 1 μ mol of inorganic P per minute from an excess of sodium phytate at pH 5.5 and 37°C.

TABLE 2. Ingredients and calculated nutrient composition of yellow dent corn (YDC), high available phosphorus corn (HAPC), phytase (PHYT), and HAPC and PHYT combination (H&P) diets

Ingredient %	0 to 21 d				21 to 42 d			
	YDC	HAPC	PHYT	H&P	YDC	HAPC	PHYT	H&P
YDC	56.11	0.00	56.52	0.00	62.40	0.00	62.73	0.00
HAPC	0.00	52.90	0.00	53.29	0.00	58.53	0.00	59.22
Soybean meal, 48%	34.65	36.32	34.57	36.25	29.61	31.47	29.54	31.40
Poultry fat	4.73	6.46	4.58	6.33	3.99	5.92	3.91	5.78
Dicalcium phosphate	2.38	1.90	1.84	1.36	1.84	1.30	1.30	0.76
Limestone	1.09	1.37	1.40	1.68	1.14	1.46	1.45	1.77
Sodium chloride	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Vitamin and mineral premix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.22	0.24	0.22	0.23	0.21	0.22	0.21	0.22
Coban 60 ²	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Phytase ³	0.00	0.00	0.05	0.05	0.00	0.00	0.05	0.05
Nutrient composition (calculated)								
CP	22.20	22.20	22.20	22.20	20.38	20.38	20.38	20.38
Ca	0.95	0.95	0.95	0.95	0.85	0.85	0.85	0.85
Available P	0.45	0.45	0.35	0.35	0.35	0.35	0.25	0.25
Total P	0.82	0.72	0.72	0.62	0.70	0.60	0.60	0.50
ME, Kcal/kg	3,141.60	3,141.60	3,141.60	3,141.60	3,174.60	3,174.60	3,174.60	3,174.60
Arginine	1.27	1.33	1.27	1.33	1.09	1.15	1.08	1.15
Lysine	1.22	1.25	1.22	1.25	1.08	1.12	1.08	1.11
Met+cys	0.94	0.94	0.94	0.94	0.88	0.88	0.88	0.88
Sodium	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19

¹The vitamin and mineral premix included per kilogram of diet: vitamin A (vitamin A acetate) 3,859 IU; cholecalciferol 1,100 IU; vitamin E (source unspecified) 5 IU; menadione, 0.5 mg; B₁₂, 5.5 µg; choline, 190 mg; riboflavin, 2.5 mg; niacin, 17 mg; D-biotin, 0.03 mg; pyridoxine, 0.5 mg; ethoxyquin, 14 mg; manganese, 28 mg; zinc, 25 mg; iron, 14 mg; copper 3.5 mg; iodine, 0.5 mg; selenium, 0.1 mg.

²Elanco Animal Health, division of Eli Lilly and Co., Indianapolis, IN. Provided 132 g monensin per kilogram as monensin sodium.

³Natuphos, BASF Corporation, Mt. Olive, NJ.

Ross⁵ broilers were obtained from a commercial hatchery and reared in 32, 1.52-m × 2.44-m pens. Each pen was stocked with 55 chicks and had a hanging feeder and nipple waterers. Lighting was continuous at 26 lx the first 3 d; thereafter, lighting was 3.6 lx. Normal rearing procedures were followed. Mortality was recorded daily. Brooding temperature was 32°C the first week, followed by 2.8°C reductions each week until 21°C was reached at 35 d and maintained for the rest of the growout.

Before the first flock, the bedding material, kiln-dried pine shavings, was weighed into the pens and spread to a depth of 10 cm. Alum was not added to the bedding before the first flock, which doubled for flock 1 the number of replications per diet and provided no main unit treatment effect. Alum was added to the litter at 0.091 kg/bird prior to flocks 2 and 3. Since excessive cake was not a problem, no caked litter was removed from pens. After the birds were removed (between trials), litter samples were taken as described below; the cake was broken and mixed prior to alum addition. The alum was spread by hand and stirred into the litter.

Litter Analysis

Subsamples of litter were taken from five locations in each pen and placed in one common container for mixing prior to pH and moisture analysis. Litter samples were dried at 80°C for 48 h and ground to 1 mm. Dried, ground

samples were extracted with deionized water (1:15 liter:water ratio), shaken for 30 min then filtered through a 2V Whatman brand filter in which wP was analyzed using a Thermo Jarrell-Ash inductively coupled plasma spectrophotometer (Sistani et al., 2001). Total litter P was determined using the spectrophotometer after dry ashing 0.5 g litter in a muffle furnace at 500°C for 4 h followed by dissolution of the ash in a mixture of HCl and H₂SO₄ (Southern Cooperative Series, 1983).

Statistical Analysis

A detailed description of the experimental design is given in the general procedures section. Interactions between and among the main and subunit effects were scrutinized. Analysis used the mixed procedure model of SAS (SAS Institute, 2000). Litter nutrient values, tP and wP, were analyzed on a trial by trial basis. Body weights, feed to gain (F:G), and mortality at 42 d were analyzed combining trials 2 and 3 to increase the power of the tests. Prior to analysis, square root transformation was utilized on mortality data (presented as percentage). Means were compared, and significance was declared based on least significant difference at probability, $P \leq 0.05$.

RESULTS AND DISCUSSION

Broiler Weight and Feed Conversion

Mean 42 d broiler BW for trials 2 and 3 ranged from 2,113 g to 2,212 g (Table 3). Phytase supplementation

⁵Aviagen North America, Huntsville, AL.

TABLE 3. Effect of alum and no alum treatments combined with yellow dent corn (YDC), high available phosphorus corn (HAPC), phytase (PHYT), and HAPC and PHYT combination (H&P) diets on broiler body weights, feed:gain, and mortality at 42 d

Item	No alum				Alum			
	YDC	HAPC	PHYT	H&P	YDC	HAPC	PHYT	H&P
Body weight, g								
Trial 1	2,400	2,399	2,478	2,456	—	—	—	—
Trial 2	2,221	2,222	2,250	2,234	2,208	2,185	2,253	2,225
Trial 3	2,113	2,081	2,174	2,093	2,019	2,096	2,102	2,048
Mean ¹	2,167 ^{ab}	2,152 ^{ab}	2,212 ^a	2,163 ^{ab}	2,113 ^b	2,140 ^{ab}	2,178 ^{ab}	2,136 ^b
3 to 6 wk Feed:gain								
Trial 1	1.76	1.71	1.72	1.69	—	—	—	—
Trial 2	1.73	1.68	1.72	1.68	1.72	1.68	1.71	1.66
Trial 3	1.84	1.79	1.82	1.78	1.85	1.77	1.75	1.77
Mean ¹	1.78 ^a	1.75 ^{ab}	1.76 ^{ab}	1.73 ^b	1.78 ^a	1.72 ^b	1.74 ^{ab}	1.71 ^b
Mortality, %								
Trial 1	5.8	2	3.8	4.3	—	—	—	—
Trial 2	1.6	2	7.6	4	5	1.6	2	3.6
Trial 3	44.6	55.6	44.6	47	42.6	46	41.6	56

^{ab}Data are from trials 2 and 3 only; means with a common letter do not meet significance criteria ($P \leq 0.05$).

¹There were eight observations per mean.

improved BW with the no alum-PHYT treatment performing best, but this treatment was not significantly different than any of the other no alum diet combinations or the alum-PHYT and alum-HAPC treatments. Other works using phytase supplementation and graded levels of dietary P to elicit a response have reported increases in body weights for birds to 21 d (Qian et al., 1996; Yi et al., 1996). Yan et al. (2000) saw significantly improved BW at 56 d with phytase supplementation added to diets containing -0.15% NRC aP. However, at the NRC requirement, they found no improvement with phytase addition and no difference between YDC and HAPC at any age. With some levels of dietary aP (also reported as nonphytate P) below NRC requirements, Ibrahim et al. (1999) reported an overall average 42 d weight of 2,100 g. The research presented here found alum treatments with YDC and H&P had the lowest mean body weights but were not significantly different than any of the other dietary treatments except for the no alum-PHYT. Field trials with alum litter treatment and YDC diets have shown heavier birds with alum treatment (Moore et al., 1999). It is important to note that all pens shared a common atmosphere and that research pen studies will not produce the magnitude of ammonia experienced in a commercial house. Therefore, it would be incorrect to assume that alum would improve performance as it has been shown to do in full-scale operations.

Feed-to-gain data are also reported in Table 3. When compared with YDC diets, F:G was improved by feeding the HAPC, PHYT, and H&P diets, with the best F:G observed in the birds receiving the H&P diet. Addition of alum to the litter had no overall significant effect on F:G.

Mortality

Ibrahim et al. (1999) reported diet dependent mortality, but their objective was to look at various levels of dietary P to determine the point at which performance was ade-

quate and fecal P minimal. The current work looked at diets containing HAPC and phytase that meet NRC requirements and how these affected broiler litter tP and wP with and without alum inclusion. Percent mortality per trial is reported in Table 3. Data from the three trials for this repeating-type parameter were combined for analysis. There were no reportable differences due to any alum or dietary treatment. Due to a spike in humidity and temperature, there was a significant mortality event the day prior to ending the third trial as evidenced by the alarmingly large numbers (41 to 56%). Though the event was unfortunate, it may suggest that mortality was not preferentially affected by any of the treatments under the severe stress conditions. In the Yan et al. (2000) trial that evaluated corn source and phytase addition with NRC, -0.075% NRC, and -0.15% NRC nonphytate P, mortality in the later stages was exacerbated by heat stress especially at the -0.15% NRC level. Mortality was decreased by the addition of phytase to the diets.

Litter Phosphorus

Dietary available P was calculated to meet NRC (1994) broiler requirements of 0.45% for 0 to 21 d and 0.35% for 22 to 42 d of age. Total litter P increased with successive flocks as expected; these data are reported in Table 4 on a gram per kilogram basis. For the first flock, tP decreased with dietary treatment as YDC (100%), HAPC (-23%), PHYT (-25%), and H&P (-43%). All subsequent discussion using percentage assumes the no alum-YDC values for a particular trial to be 100%. Phytase inclusion in broiler diets, when formulations account for the phytate P released by the enzyme, result in reduced fecal P (Ibrahim et al., 1999), substantiating the results given here. With HAPC diets, lower P excretion is seen because less phytate P enters the broiler system (Waldroup et al., 2000; Yan et al., 2000), a result that we have also observed.

TABLE 4. Effect of alum or no alum treatments combined with yellow dent corn (YDC), high available phosphorus corn (HAPC), phytase (PHYT), and HAPC and PHYT combination (H&P) diets on total broiler litter phosphorus (tP) and litter water-soluble phosphorus (wP)

Dietary treatment	Trial 1 ¹		Trial 2 ²		Trial 3 ²	
	No alum	Alum	No alum	Alum	No alum	Alum
tP(g/kg)						
YDC	12.2 ^a		15.5 ^a	14.4 ^b	17.1 ^a	16.5 ^a
HAPC	9.4 ^b		13.3 ^c	12.1 ^d	15.3 ^b	13.1 ^c
PHYT	9.1 ^b		13.3 ^c	13.8 ^{bc}	14.8 ^b	14.4 ^b
H&P	6.9 ^c		9.7 ^e	9.6 ^e	12.4 ^c	11.0 ^d
LSD	(1.06)		(0.73)		(1.04)	
Mean ³			12.9 ^x	12.5 ^y	14.9 ^x	13.8 ^y
LSD ⁴			(0.37)		(0.52)	
wP(g/kg)						
YDC	2.17 ^b		2.41 ^a	1.01 ^b	3.42 ^a	2.01 ^{bc}
HAPC	1.52 ^c		2.34 ^{ab}	1.21 ^b	3.22 ^a	1.33 ^d
PHYT	2.85 ^a		2.86 ^a	1.98 ^{ab}	3.47 ^a	2.24 ^{bc}
H&P	1.54 ^c		1.79 ^b	1.30 ^b	2.50 ^b	1.63 ^{cd}
LSD	(0.34)		(0.44)		(0.60)	
Mean ³			2.35 ^x	1.37 ^y	3.15 ^x	1.81 ^y
LSD			(0.22)		(0.30)	

^{a-e}Within trial, means with different letter differ significantly ($P \leq 0.05$).

^{x,y}Within trial, different letters indicate significant ($P \leq 0.05$) difference in overall alum vs. nonalum treatments.

¹There were eight observations per mean in trial 1.

²There were four observations per mean in trials 2 and 3.

³There were 16 observations per overall mean comparing alum vs. no alum treatments in trials 2 and 3.

⁴LSD = least significant difference.

At the end of trial 2, the mean tP was significantly lower for the alum treatments. While alum addition should not affect tP, the difference may be explained by the total litter weigh-out after the final trial (data not shown). In the no alum treatments, the total bedding/manure mix averaged 7.2 kg heavier than the alum treatments for a particular diet. This corresponds to the generally greater body weights of the no alum treatments and is also indicative of greater P deposition on the litter because of more manure excreted by the larger birds. In runoff studies for standard (YDC diet) litter and alum use, research has shown up to a 72% reduction in tP runoff over a 3-yr watershed study (Moore et al., 1999). The no alum-YDC diet produced the most tP in the second trial, 15.5 g/kg, and again in the third trial, 17.1 g/kg. The alum-YDC treatment resulted in 16.5 g/kg tP in the third trial and was not different than the no alum-YDC. Vetesi et al. (1998) reported a 15% reduction in P excretion using phytase at 600 phytase units/kg feed. The PHYT diet in trial 2 averaged a 13% reduction in tP and a 15% decrease in trial 3. The HAPC diets differed significantly with alum addition. With no alum addition, a 14% tP reduction was seen in the second grow-out; with alum the reduction was 22%. No alum-HAPC tP was down 11% in the third trial with a corresponding alum-HAPC reduction of 23% tP. The alum-combination diet, H&P, produced the least tP in the last trial with a 36% reduction.

High wP concentrations have been observed on fields where poultry litter has been applied (Moore et al., 1995b). Soluble P losses in runoff normally occur only in soils that have become excessive in P and are most commonly associated with erosion followed by situations in which soils have been amended with animal manure (Williams

et al., 1999). Results, including wP determination with alternative diets, HAPC and PHYT, and alum litter treatment, are presented in Table 4. For the first trial, wP in the litter was greatest for PHYT (2.85 g/kg or +31%), followed by YDC (2.17 g/kg), and then together by H&P and HAPC diets (1.5 g/kg or -30%). The second trial wP was greatest for no alum-PHYT (+19%), which was not significantly different than no alum-YDC or HAPC (-3%) or alum-PHYT (-18%). In trial three, when there was no alum addition to the YDC, PHYT, or HAPC dietary treatments, the wP was greatest. For all diets in the final growout, alum reduced litter wP from 35 to 61%. It would be incorrect to say that there is no difference in the no alum-YDC, PHYT, and HAPC dietary treatments, based on the null hypothesis testing employed. However, precision is fairly high based on the least significant difference, imparting confidence to the conclusions. Without alum addition, the results show that wP is not reduced significantly by the use of HAPC and that phytase supplementation did not decrease wP.

Many benefits can be enjoyed with the use of alternative feedstuffs and supplements, but other options, such as tailoring broiler P requirements to growth stage (Stillborn, 1998; Waldroup, 1999) and use of effective litter treatments like alum, will be required to minimize excess litter P. Using HAPC and alum were mutually beneficial for reducing wP. Feed conversion was best for the combination diet (H&P) as well as the HAPC diet. Phytase supplementation improved BW; however, more investigation of P speciation and litter management effects are warranted.

In conclusion, the modified broiler diets discussed are generally considered acceptable for bird performance heretofore the primary consideration of the industry, but

their ultimate effect on the environment must also be measured. The not-to-distant future holds comprehensive regulation of outputs (cake/litter, aerial components) from broiler as well as other animal production facilities, requiring those industries to use a systems approach in becoming as efficient and responsible as possible. The results of this work relating to wP in broiler litter imply that the use of phytase enzyme in a normal corn diet can not in itself address excess P in broiler litter. Although tP is reduced, wP is not. The combination of HAPC and phytase in a diet appear promising for reducing the amount of wP in litter compared with that of a normal corn diet. Moreover, use of alum litter treatment was shown to reduce wP in litter for all dietary formulations.

ACKNOWLEDGMENTS

High available phosphorus corn was contributed by Pioneer Hi-bred International, Inc., Johnston, Iowa. Phytase and phytase activity assays were donated by BASF Corporation, Mt. Olive, NJ. Dedication of the technical staff at the USDA-ARS Poultry and Waste Management and Forage Research Units is gratefully acknowledged. Critical review of the manuscript by Hart Bailey, Timothy Chamblee, and Larry Oldham is appreciated.

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